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# Research Article
# Antioxidant Effects of Tomato Juice on Reducing Serum Malondialdehyde Levels in Menopausal Rats

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## Abstract

**Background and Objective:** Menopause is characterized by a loss in reproductive ability. Aging is a progressive loss of biochemical and physiological processes after the reproductive phase, which is affected by oxidative stress and free radicals. Tomatoes contain antioxidants. This study aimed to determine the antioxidant effects of tomato juice on serum malondialdehyde levels in menopausal rats. **Materials and Methods:** This was an experimental study. Twenty-four female *Sprague-Dawley* rats were divided into four groups. The negative control was a group of sham procedure rats that were treated with distilled water. The positive control was a group of bilateral ovariectomized rats that were treated with distilled water. The first treatment was a group of bilateral ovariectomized rats that were treated with tomato juice at the dosage of 11 g/200 g body weight day$^{-1}$. The second treatment was a group of bilateral ovariectomized rats that were treated with tomato juice at the dosage of 15 g/200 g body weight day$^{-1}$. Treatment was given for 28 days. **Results:** The highest serum malondialdehyde level was found in the positive control group (126.70±24.76 ng mL$^{-1}$). The serum malondialdehyde level in the first treatment group (120.40±39.81 ng mL$^{-1}$) was lower than that in the positive control group (126.70±24.76 ng mL$^{-1}$). The lowest serum malondialdehyde level was in the second treatment group (81.33±10.51 ng mL$^{-1}$). There was a negative correlation between the amount of tomato juice and the serum malondialdehyde level (b = -0.573). **Conclusion:** Tomato juice, as an antioxidant, can reduce serum malondialdehyde levels in menopausal rats. The higher the dose of tomato juice, the lower the serum malondialdehyde level.

Key words: Menopause, rats, serum malondialdehyde levels, tomato juice, vitamin C

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Menopause is a natural period characterized by the loss of reproductive ability. More than half of postmenopausal women experience varying levels of menopausal symptoms and anxiety because of the aging process[1,2].

Aging is defined as a progressive loss of biochemical efficiency and physiological processes after the reproductive phase of life[3]. The contribution of free radicals to the aging process occurs starting at the beginning of life and increases with aging. Aging is an accumulation of progressive changes associated with increased susceptibility to disease and the amount of damage caused by continuous free radical reactions in cells and tissues. The aging process is characterized by the loss of progressive physiological integrity, which triggers dysfunction and death[4,5].

Free radicals are highly reactive and can cause chemical changes and damage various components, such as carbohydrates, nucleotides, lipids and proteins. Damage to these important molecules will play a role in degenerative changes in the aging process. The body produces antioxidants, such as glutathione peroxidase, catalase and superoxide dismutase, so that the body can reduce free radicals. Increasing age causes the number of free radicals to increase so that the natural antioxidants are insufficient. The imbalance between produced free radicals and natural antioxidants causes the formation of reactive oxygen species (ROS)[3,6].

The reactivity of ROS causes damage to the molecular structure of cell membranes. Cell membranes are sensitive to free radicals. The molecular structure of cell membranes comprises cholesterol, phospholipids and glycolipids. Reactivity of ROS results in the formation of many lipid peroxides. Lipid peroxidation will cause the release of malondialdehyde (MDA)[7,8].

Antioxidants are molecules that are able to deactivate or stabilize free radicals before attacking cells and inhibit or delay oxidation. Antioxidants can play a role in reducing the rate of change because of aging. Antioxidants have preventive and protective functions in age-related diseases[9]. Endogenous antioxidants may be inadequate under conditions of increased oxidative stress. Thus, food antioxidants are required to support optimal cellular function[3].

Tomatoes contain vitamin C, β-carotene and lycopene, which have antioxidant effects. Vitamins C and E are secondary antioxidants that can bind free radicals and prevent chain reactions. Several studies have used vitamin C as a positive control to determine antioxidant activity[10]. Lycopene is a powerful antioxidant and can react with free radicals. Nutrients in tomatoes can work to prevent and fight diseases associated with oxidative stress through various mechanisms of antioxidant action[11,12].

The aim of this study was to assess the effects of tomato juice as an antioxidant on serum MDA levels as a marker of oxidative stress in menopausal rats.

## MATERIALS AND METHODS

**Study design:** This was a true experimental study. This study used healthy female *Sprague-Dawley* rats, aged 4 months that weighed 150-200 g. The animals were supplied by the Integrated Research and Testing Laboratory, Universitas Gadjah Mada Yogyakarta. The study material was tomatoes (*Solanum lycopersicum*), which were certified and came from plantations in Malang City. Maintenance and treatment of the rats was carried out in the animal cages of the Faculty of Veterinary Medicine, Universitas Airlangga Surabaya. The examination of serum MDA levels was carried out at the Biology Service Unit, Faculty of Science and Technology, Universitas Airlangga Surabaya.

**Preparation of tomato juice:** Tomato fruits of the same variety were picked 80-90 days after planting. Tomato juice was prepared by separating the inner tomato from the skin and seeds using an electric juicer.

**Treatment of rats:** The study began with the acclimatization of twenty-four healthy female rats for two weeks. The rats were randomly divided into four groups of equal size. The negative control (NC) was a group of sham procedure rats that did not undergo ovariectomy and were treated with distilled water. The positive control (PC) was a group of bilateral ovariectomy rats treated with distilled water. The first treatment (T1) was a group of bilateral ovariectomized rats that were treated with tomato juice at a dosage of 11 g/200 g of body weight day$^{-1}$. The second treatment (T2) was a group of bilateral ovariectomized rats treated with tomato juice at a dosage of 15 g/200 g of body weight day$^{-1}$. The condition of menopause was confirmed by vaginal swab on the 21st day after bilateral ovariectomy. Treatment was given for 28 days. Intracardiac blood sampling was carried out on the 29th day and the rats were sacrificed.

**MDA examination:** Serum MDA levels were examined by the thiobarbituric acid reactive substances (TBARS) method using the Oxytech MDA-586 assay reagent (OXIS International, Inc.). A sample of 0.05 mL taken with a pipette was put into a

tube containing 0.75 mL of phosphoric acid and 0.25 mL of thiobarbiturate acid (TBA) solution. Then, 0.45 mL of water was added and the mixture was homogenized. The samples were heated in a water bath for 60 min at a temperature of 100°C and then cooled for 1-2 h so that the temperature reached 30°C. After that, the samples were placed in a C18 Sep-Pak and 4 mL of methanol was added. Color density was observed with a spectrophotometer at a wavelength of 530 nm.

**Statistical analysis:** Statistical analysis was performed using one-way analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$. The Kolmogorov-Smirnov test found an abnormal distribution of data ($p<0.05$), so analysis continued with the Kruskal-Wallis test. The Mann-Whitney test was performed for multiple comparisons. A regression test was performed to assess the association between the dose of tomato juice and serum MDA levels.

**Ethical approval:** Ethical clearance was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga, with number KE.024.02.2018.

## RESULTS

The results showed that tomato juice can reduce serum MDA levels in menopausal rats.

As shown in Table 1, the highest serum MDA level was in the positive control group, with a value of 126.70±24.76 ng $mL^{-1}$. Serum MDA levels in the first treatment group (T1) (120.40±39.81 ng $mL^{-1}$)was lower than that in the positive control group (126.70±24.76 ng $mL^{-1}$), although still higher than that in the negative control group (86.52±41.47 ng $mL^{-1}$). The lowest mean serum MDA level was in the second treatment group (T2), with a value of 81.33±10.51 ng $mL^{-1}$.

As shown in Fig. 1, increasing the dose of tomato juice can reduce serum MDA levels. The mean serum MDA levels in the two treatment groups (T1) and (T2) were lower than that in the positive control group. The mean serum MDA level in the second treatment group (T2) was lower than that in the first treatment group (T1).

Table 2 shows the multiple comparison tests. There was a significant difference in serum MDA levels between the negative control group and the first treatment group (T1), with a $p = 0.037$. Additionally, there was a significant difference in serum MDA levels between the positive control group and the second treatment group (T2), with a $p = 0.006$. Regression tests showed that increasing the dose of tomato juice could reduce serum MDA levels, with a standard coefficient of 0.573 ($b = -0.573$).

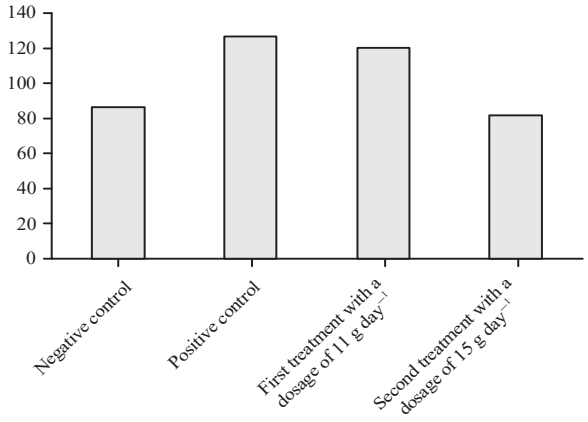


Fig. 1: Mean serum MDA levels

Table 1: Mean, standard deviation, median, minimum and maximum MDA levels

| Groups | NC | PC | T1 | T2 | p-value |
|---|---|---|---|---|---|
| Mean | 86.52 | 126.70 | 120.40 | 81.33 | |
| Median | 69.67 | 119.11 | 118.55 | 80.77 | |
| Standard deviation | 41.47 | 24.76 | 39.81 | 10.51 | 0.017* |
| Minimum | 64.11 | 94.11 | 81.89 | 70.78 | |
| Maximum | 170.78 | 156.33 | 189.67 | 96.33 | |

*Significance value of $p<0.05$ was tested with ANOVA. NC: Negative control group, PC: Positive control group, T1: First treatment group, T2: Second treatment group

Table 2: Association between the dose of tomato juice and serum MDA levels

| | p-value | | | | |
|---|---|---|---|---|---|
| Groups (MDA) | NC | PC | T1 | T2 | Standard coefficient (b) |
| NC (8.52±41.47) | - | 0.054 | 0.037[a] | 0.171 | -0.573[b] |
| PC (126.70±24.76) | | - | 0.687 | 0.006[a] | |
| T1 (120.40±39.81) | | | - | 0.054 | |
| T2 (81.33±10.51) | | | | - | |

[a]Mann-Whitney U test, [b]Regression test. NC: Negative control group, PC: Positive control group, T1: First treatment group, T2: Second treatment group

## DISCUSSION

Malondialdehyde is a final product and biological biomarker of lipid peroxidation. Malondialdehyde describes the degree of oxidative stress and tissue damage because of free radicals[7]. This study found that the highest serum MDA level was in menopausal rats treated with distilled water. This means that lipid peroxidation and free radicals increase during menopausal conditions. According to free radical theory, dysfunction in aging is triggered by physiological and biochemical changes during the aging process caused by an increase in free radicals. Oxidative stress and free radicals with genetic and environmental modifications play a role in the aging process. A continuous increase in free radicals results in an imbalance between the number of free radicals and antioxidants. This imbalance causes an increase in the formation of ROS during the aging process[3,6].

Tomato juice is an exogenous antioxidant. Antioxidants are easily oxidized. Free radicals or reactive oxygen species will oxidize antioxidants, thus protecting other molecules from damage. This study found that administration of tomato juice reduced serum MDA levels in menopausal rats. Tomatoes contain lycopene, vitamin C and vitamin E. Lycopene is a powerful antioxidant that can bind to free radicals. Vitamin C is a secondary antioxidant that has the same mechanism of action as vitamin E. Vitamins C and E can bind to free radicals and prevent chain reactions[10,11]. According to Maong *et al.*[13], tomatoes have singlet oxygen stabilizing activity. The smaller the peroxides of the tomato extract, the greater the singlet oxygen stabilizer potential.

In addition, the tomato variety, origin and juice preparation are factors that can influence a significant decrease in serum MDA levels in menopausal rats. The tomatoes used in this study were from the same plantation and were picked according to the correct time at 80-90 days. The carotenoid compound in tomatoes is lycopene. Lycopene is a red pigment in tomatoes. The lycopene content in tomatoes varies because of genetic influences, fruit maturity when picked, agronomic effects and environmental conditions during planting. Changes in pigment can assess an increased carotenoid content. An increased concentration of lycopene causes an increase in the red pigment of tomatoes. Mu'nisa[14] found that the lycopene content of tomatoes from Tana Toraja was higher than that of tomatoes from Malino in South Sulawesi.

The amount of lycopene influences the antioxidant actions of tomatoes. The higher the levels of lycopene in tomatoes, the higher the antioxidant action[14]. This study used tomato juice preparations because the preparation of tomatoes influences the lycopene content. Tomato flesh has the highest lycopene content. Crushed tomatoes are a better source of lycopene than raw tomatoes. Crushed tomatoes can release more lycopene that is easily absorbed by the body. The amount of lycopene in tomato juice can reach twice the level compared with raw or fresh tomatoes. The processing of tomatoes can affect the content of lycopene and carotenoids[15,16]. Ma'sum *et al.*[17] found that the antioxidant activity from a fresh tomato extract was greater than that in a tomato paste extract. During the heating process, vitamin C undergoes oxidation and phenolic compounds undergo chemical changes, decomposition, or the formation of protein complexes.

The content of vitamins C and E might also influence serum MDA levels in this study. Vitamins C and E are exogenous antioxidants that play an important role. Vitamin C is the main hydrophilic antioxidant and fat peroxidation inhibitor. In the membranes, these molecules rapidly decrease the number of α-tocopheroxyl radicals to regenerate α-tocopherol and inhibit free radical propagation. Vitamin E (α-tocopherol) is a major hydrophobic antioxidant in membranes and lipoproteins. Vitamin E reduces lipid peroxidation to fatty acids, which form tocopherol radicals. Tocopherol radicals are relatively stable and long-lasting until they are reduced again by vitamin C in cell membranes or lipoproteins. After reacting with tocopherol radicals, vitamin C forms a monodehydroascorbate radical and then undergoes enzymatic or nonenzymatic reduction. Enzymatic reduction is catalyzed by glutathione peroxidase to form vitamin C and oxidized glutathione. Nonenzymatic reduction occurs through the reaction of two monodehydroascorbate molecules to form ascorbate and dehydroascorbate. Both of these compounds are not free radicals[3,11].

Vitamin C in tomato juice is helpful during menopause. Vitamin C is a mediator of collagen biosynthesis. Increased collagen density can increase elasticity of the vaginal wall[18]. During the menopausal period, vaginal atrophy occurs because of epithelial thinning and decreased collagen and elastin fibers. There is a decrease in vaginal blood circulation, collagen hyalinization, elastin fiber fragmentation and the proliferation of connective tissue in the vagina[19,20].

Synthesis of collagen from fibroblasts requires vitamin C[21]. Free radicals can affect collagen metabolism. Free radicals can also destroy collagen directly or induce matrix metalloproteinases (MMPs), which can breakdown collagen type-1 and type-4, resulting in decreased collagen products[22]. According to Wahyono[23], tomato extract administration at high doses can increase collagen type-1 and decrease MMP-1 and MMP-3 on the skins of rats experiencing aging. Saimin *et al.*[24] found that the administration of tomato juice reduced the expression of MMP-2 and increased the expression of collagen type-1 in the vaginal walls of menopausal rats.

This study shows that administration of tomato juice at the dosage of 11 g $day^{-1}$ can reduce serum MDA levels in menopausal rats. Increasing the dose to 15 g $day^{-1}$ can significantly reduce serum MDA levels, reaching a normal level. There is a negative correlation between the dose of tomato juice and serum MDA levels, which means that increasing the dose of tomato juice will reduce serum MDA levels. This study shows that tomato juice, which has lycopene, β-carotene, vitamin C and vitamin E, is a powerful antioxidant that can reduce serum MDA levels during menopausal conditions.

## CONCLUSION

Tomato juice, as an antioxidant, can reduce serum MDA levels in menopausal rats. There is a negative correlation between the dose of tomato juice and serum MDA levels with a standard coefficient of b = -0.573. The higher the dose of tomato juice, the lower the serum MDA level. Thus, tomato juice has the potential to be developed as an antioxidant in menopausal women.

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